His Tag ELISA Detection Kit

Technical Manual No. TM0635 **Version** 08292012

Product Name	**Cat.No**	**Size**
His Tag ELISA Detection Kit	L00436	1 plate (8 wells x 12 strips)

The product is used for rapid and high throughput detection of His-tagged proteins.

The operator should read technical manual carefully before using this product.

For research use only. Not for use in diagnostic procedures.

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-free Tel: 1-877-436-7274 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

Contents

I. Description 2
II. Key Features 3
III. Kit Content 3
IV. Storage 3
V. Reagents/Equipments Required But Not Supplied 3
VI. Instruction for Use 4
1. Sample Preparation 4
2. Reagent Preparation 4
3. His-Tag Plate Preparation 4
4. Test Procedure 5
His-Tagged Protein/Anti-His Monoclonal Antibody Incubation 5
Antibody Tracer Incubation 5
Substrate Reaction and Absorbance Measurement 5
VII. Assay Procedure Summary 6
VIII. Typical Assay Data 7
IX. Reagent Compatibility 7
X. Troubleshooting 8
XI. Related Products 8
XII. Plate Layout 10

I. Description

His tag is successive histidine (H) residues and there are mainly three forms: HHHHHH (6 x His), HHHHH (5 x His), HHHH (4 x His). Due to its small size, less interfere in protein folding, weak immunogenicity, His tag is the most dominant tag, which is widely used in recombinant protein expression. The DNA sequence which codes for His tag, is usually constructed at N-terminus or C-terminus of variety of expression plasmids. Since His tag has high affinity for Ni^{2+} ions, His-tagged proteins can be easily purified from bacteria, yeast and mammalian cell expression system by Ni^{2+}-resin chromatography. Anti-His tag antibody is a useful tool for the identification of His-tagged proteins with various methods such as western blot, immunoprecipitation and flow cytometry.

GenScript His Tag ELISA Detection Kit, a 1.5 hour competition ELISA**,** is developed for rapid and high throughput detection of His-tagged proteins. There are several potential usages:

- Quickly identify the presence of His-tagged proteins in samples.
- Optimize protein expression by monitoring the His-tagged proteins level.
- High throughput screening of stable cell lines expressing His-tagged proteins.

This kit is based on competitive ELISA method. His Tag Plate in the kit is a 96-well microtiter plate coated with a His-tagged protein with the molecular weight of 12.7 kDa. The plate, which comprises of 8 wells x 12 strips, is demountable. When Anti-His Monoclonal Antibody and His-tagged proteins are added to the well , the coated His-tagged proteins compete with His Tag Standard (His-tagged protein with the molecular weight of 11.3 kDa) in solution or His-tagged protein in sample to interact with Anti-His Monoclonal Antibody. The higher the concentration of the His-tagged protein in solution is, the less the antibody bound to the plate will be. The Anti-His Monoclonal Antibody used in the kit is a mouse anti His tag monoclonal antibody (GenScript A00186). Antibody Tracer, which is a horseradish peroxidase (HRP) conjugated Goat anti mouse IgG, is used for enzyme reaction. His-tagged protein, Anti-His Monoclonal Antibody and antibody tracer form a complex. Other unbound molecules can be removed by washing solution. The antibody tracer reacts with TMB substrate to develop blue product that turns yellow immediately when the Stop Solution is added, which can be measured by microplate reader at 450 nM.

In optimized test condition, each absorbance value is indicated to the individual His-tagged proteins amount in solution. His-tagged protein standards of known concentration and the corresponding absorbance values are used to construct a standard curve. With the standard curve, His-tagged protein amount present in the unknown sample can be calculated by transforming its absorbance value.

II. Key Features

Features	Specifications
Sensitivity	1 ng/ml His-tagged proteins
Detection Range	1 ng/ml~729 ng/ml
Test Samples	N-terminal/C-terminal/internal His-tagged proteins
	4 x His/5 x His/6 x His-tagged proteins
	Mammalian, yeast and bacteria cell lysates or cell supernatant
Conveniency	Provide all reagents required for test
	Complete test within 1.5 hours
Reagent Compatibility	Tolerable with various reagents at certain concentration (see Table 3)

III. Kit Content

The kit provides all reagents and solutions required for His-tagged protein detection.

- His Tag Standard Stock can be used to prepare His Tag Standard with Assay Diluent as an alternative.

Table 1 Kit components

Component	Quantity	Part. No
His Tag Plate	1 plate (8 wells x 12 strips)	436-80
Anti-His Monoclonal Antibody	6 ml	436-20
Antibody Tracer	12 ml	436-30
His Tag Standards (0,1,3,9,27,81,243,729 ng/ml)	2 ml per each standard	436-11,12,13,14,15,16,17,18
Assay Diluent	60 ml	436-60
20 × Wash Solution	40 ml	436-70
TMB Substrate	12 ml	436-40
Stop Solution	6 ml	436-50
His Tag Standard Stock (10 μg/ml)	500 μl	436-10
Plate Sealer	2	N/A
User Manual	1	N/A

IV. Storage

The unopened kit is stable for at least 12 months if stored at 2-8 °C and the opened kit may be stable for up to 1 month at 2-8 °C. Do not freeze the kit.

V. Reagents/Equipments Required But Not Supplied

Microtiter plate reader capable of measuring absorbance at 450 nm

Automated microplate washer

Deionized or distilled water

Graduated cylinder to prepare Wash Solution

Plastic container to store Wash Solution

Tubes to aliquot and dilute samples

Precision pipettes to deliver 10 µl, 100 µl, 200 µl and 1000 µl content

10 µl, 100 µl, 200 µl and 1000 µl pipette tips

Multichannel pipettor

Disposable reagent reservoirs

Paper towel

Laboratory timer

Refrigerator to store samples and kit components

VI. Instruction for Use

1. Sample Preparation

When preparing samples for ELISA assay, several key factors should be considered:

- Minimize concentration of certain reagents in the sample. For some reagents may interfere with test result, read the section of reagents compatibility carefully.
- Samples should not contain any particles/precipitates. Filter the sample or centrifuge as necessary to remove insoluble materials.
- For best results, the sample should be adjusted to neutral pH (6.8-7.4).

2. Reagent Preparation

- If any precipitate is found in the 20 × Wash Solution, incubate the bottle in water bath (up to 50 °C) with occasional mixing until all the precipitate disappears.

1 x Wash Solution: Dilute *20 × Wash Solution* by 1:19 v/v with deionized or distilled water. For example, dilute 40 ml of *20 × Wash Solution* with 760 ml of distilled water to make 800 ml of 1 × *Wash Solution*. The prepared 1 × *Wash Solution* can be stored at 2-8 °C for at least one month.

3. His-Tag Plate Preparation

- It is recommended that all standards and samples are prepared in duplicate.
- Make sure the strips are tightly snapped in the plate frame.

1. Count the strips for the assay.
2. Leave the unused strips in the foil pouch and store at 2-8 °C.

4. Test Procedure

- All reagents in the kit and test samples should be equilibrated to room temperature before test.
- Preliminary experiment should be performed to optimize sample dilution. Use Assay Diluent for sample dilution.
- The test should not be performed over 25 °C.

His-Tagged Protein/Anti-His Monoclonal Antibody Incubation

1. Add 50 µl of *His Tag Standards* or samples containing His-tagged proteins to each well of His Tag Plate.
2. Add 50 µl of *Anti-His Monoclonal Antibody* to all the wells.
3. Cover the plate with *Plate Sealer* and incubate at room temperature (20-25 °C) for 30 minutes.
4. Wash the plate with 260 µl of *1 x Wash Solution* four times.
5. Pat the plate on paper towel to remove residual liquid in the wells.

Antibody Tracer Incubation

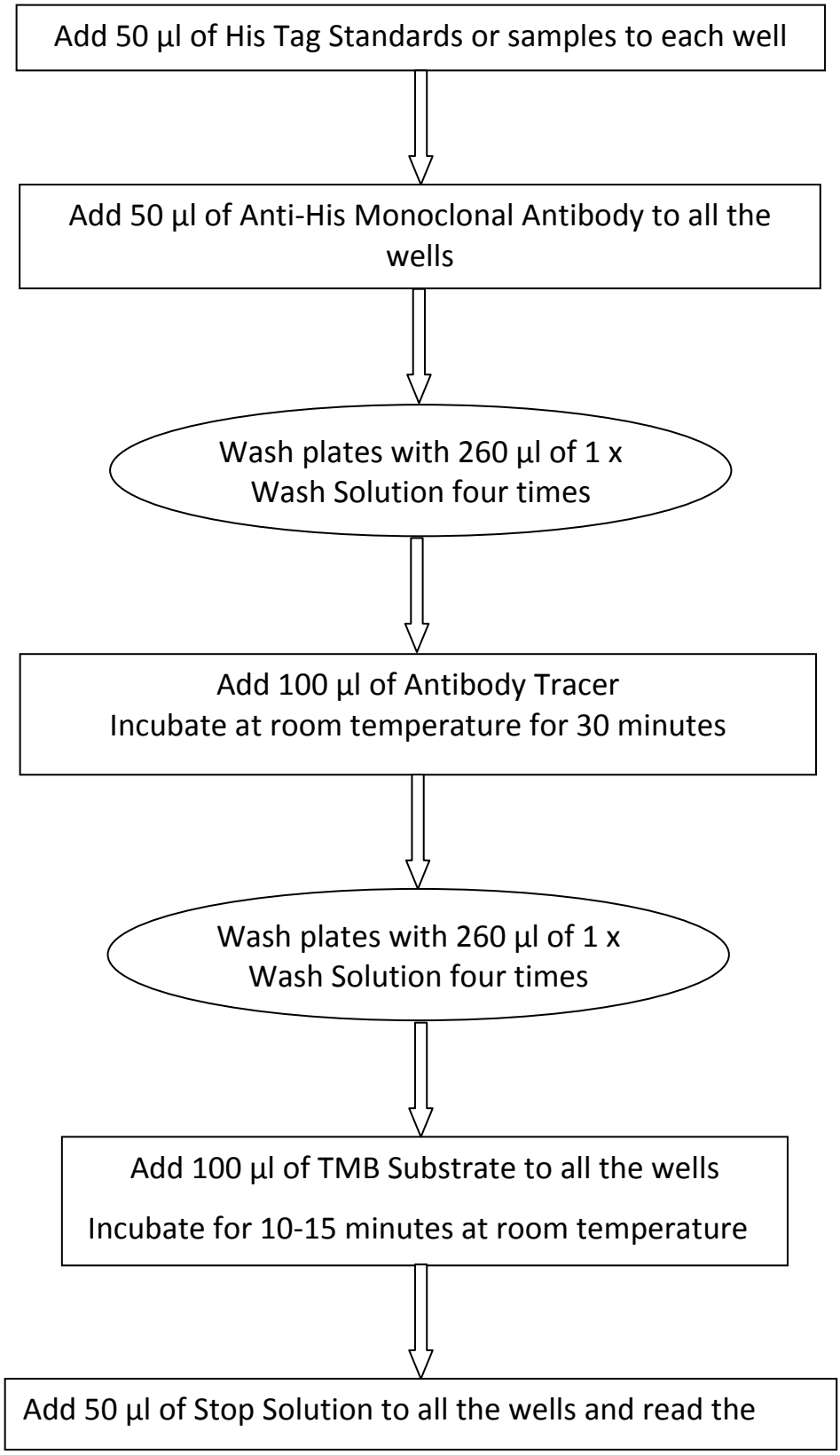
6. Add 100 µl of *Antibody Tracer* to all the wells.
7. Cover the plate with *Plate Sealer* and incubate at room temperature for 30 minutes.
8. Wash the plate with 260 µl of *1 x Wash Solution* four times.
9. Pat the plate on paper towel to remove residual liquid in the wells.

Substrate Reaction and Absorbance Measurement

- After adding Stop Solution, solution in the wells turns yellow.
- To ensure test stability, read the plate at 450 nm immediately after adding Stop Solution.
- If the sample is diluted, multiply the interpolated value by the dilution factor to calculate the amount of His-tagged proteins in sample.

10. Add 100 µl of *TMB Substrate* to all the wells and incubate at room temperature for 10-15 minutes.
11. Add 50 µl of *Stop Solution* to all the wells to stop the enzyme reaction.
12. Read absorbance of the plate on microplate reader at 450 nm.
13. Generate a standard curve by plotting the absorbance on the vertical axis versus the His-tagged standard concentration on the horizontal axis.
14. The amount of His-tagged protein in sample is determined by extrapolating its OD value to the standard curve.

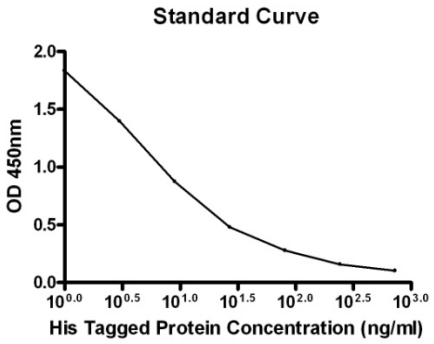
VII. Assay Procedure Summary


Add 50 µl of His Tag Standards or samples to each well
Add 50 µl of Anti-His Monoclonal Antibody to all the wells
Wash plates with 260 µl of 1 x Wash Solution four times
Add 100 µl of Antibody Tracer
Incubate at room temperature for 30 minutes
Wash plates with 260 µl of 1 x Wash Solution four times
Add 100 µl of TMB Substrate to all the wells
Incubate for 10-15 minutes at room temperature
Add 50 µl of Stop Solution to all the wells and read the

VIII. Typical Assay Data

The standard curve was provided for demonstration only. It should be prepared each time an assay is performed.

Figure 1. Standard curves of assay results Table 2 .Example of assay results



Conc. of His Tag Standard		OD 450		
(ng/ml)	(pmol/ml)	Duplicate 1	Duplicate 2	Average
0	0	2.177	2.183	2.180
1	0.088	1.833	1.831	1.832
3	0.265	1.374	1.418	1.396
9	0.796	0.825	0.922	0.874
27	2.389	0.516	0.444	0.480
81	7.168	0.266	0.289	0.278
243	21.504	0.158	0.157	0.158
729	64.513	0.105	0.101	0.103

IX. Reagent Compatibility

Reagents in test sample may interfere with test result. Common detergents and denaturants have been tested for compatibility and interference with the assays.

Table 3. Reagent compatibility

Reagent	Recommended Use
Triton X-100	≤ 1%
Imidazole	≤ 125 mM
Guanidine HCl	≤ 30 mM
Urea	≤ 0.5 M
Deoxycholic Acid	≤ 1%
SDS	≤ 0.07%
EDTA	≤ 10 mM
β-ME	≤ 160mM
DTT	≤ 20 mM
Tris (pH=7)	≤ 6.5 mM
CHAPS	≤ 5%
Tween-20	≤ 1%
Glycerol	≤ 1%
TBS	Fully compatible
PBS	Fully compatible
RIPA Lysis Buffer	Fully compatible

X. Troubleshooting

Problem	Probable Cause	Solution
Poor Precision	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Wells have been scratched with pipette tip or washing needles	Dispense and aspirate solution into and out of wells with caution
	Particulates are in the samples	Remove any particulates by centrifugation prior to the assay
	Pipette error	Check pipette calibration and repeat assay
	Components are used from other lots or sources	Never substitute any components from another kit
	Components are not brought to room temperature prior to the assay	Repeat assay with components that have been equilibrated to room temperature
Weak/No Signal	TMB Substrate are not added or were added at the wrong time	Follow the manual to add the TMB Substrate
	Antibody Tracer is not added, or was added at the wrong time	Follow the manual to repeat the assay
	TMB Substrate has been contaminated	Use new TMB Substrate
	Do not add the proper volumes of reagents	Repeat the assay with the required volumes in manual
	Do not incubate the plate for proper time or at proper temperature	Follow the manual to repeat the assay
	Do not read the plate immediately after Stop Solution was added	Read the plate within 10 minutes
High background	Plate is not washed properly	Make sure the wash apparatus is functioning properly. Make sure wash solution is removed before adding substrate
	TMB Substrate has been contaminated	TMB Substrate is very light sensitive and must be protected from direct light. Use new TMB Substrate with same Lot
	Evaporation of wells during incubations	Perform incubation steps with Plate Sealer in the assay
	Incorrect incubation times and/or temperatures	Follow the manual to repeat the assay

XI. Related Products

- His Tag Antibody, pAb, Rabbit L00411
- Mouse Anti-His mAb MagBeads L00275
- His Tag Antibody Plate L00440
- THE™ His Tag Antibody, mAb, Mouse A00186
- THE™ His Tag Antibody [HRP], mAb, Mouse A00612
- THE™ His Tag Antibody [Biotin], mAb, Mouse A00613
- THE™ His Tag Antibody [FITC], mAb, Mouse A01620

Limited Use Label license: This product may be the subject of one or more patents filed by GenScript Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer may not sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for any commercial purposes. For commercial use, please contact GenScript at product@genscript.com.

GenScript USA Inc.

860 Centennial Ave.,

Piscataway, NJ 08854

Tel: 1-877-436-7274, 1-732-885-9188

Fax: 732-210-0262, 732-885-5878

Email: product@genscript.com

Web: www.genscript.com

XII. Plate Layout

Use this plate layout to record standards and samples assayed.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Notes:

Use this plate layout to record standards and samples assayed.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Notes: